IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: William Velander *et al.*
Serial No.: 10/049,849 Group No.: 1632
Filed: 06/27/2002 Examiner: Hama, J.
Entitled: **Transgenic Prothrombin And Related Prothrombin Precursors**

SECOND DECLARATION OF DR. WILLIAM VELANDER UNDER 37 CFR § 1.132

Mail Stop –Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Examiner Hama:

I, Dr. William Velander, under penalty of perjury, state that:

1. I am a co-inventor of the United States patent application captioned above.
2. I am qualified as an expert in the field of protein expression from transgenic mammals.
3. Recent research conducted under my direction has provided data relevant to the above captioned patent application. A transgenic pig (Grandma) was created in accordance with the teachings of the above captioned application that secretes milk having at least 1.5 mg/ml of a human prothrombin amino acid sequence.
4. The prothrombin assay was conducted by first collecting a daily milk sample by inducing lacation. The sample was then processed and diluted by adding aminocaproic acid and ethylene diamine tetraacetic acid. A aliquot of each milk sample collected on Day 5, 10, 15 and 20 following the lactation induction protocol were used to generate a Western Blot immunoassay (nonreduced 12% Bis-Tris Gel) where prothrombin was detected using a horseradish peroxidase

labeled anti-prothrombin polyclonal antibody (US Biologicals, P9115-16A, Lot # L9080754). See, Figure A.

5. Each gel band was analyzed using densitometry using Adobe Photoshope image analysis that measures absolute intensity in terms of "mean x the total number of pixels". These data were compared against a standard curve generated from a parallel analysis of plasma derived factor II/IIa as a standard reference at three different concentrations. See, Figure B. Sample dilution correction factors were determined in terms of the contribution of raw milk in each assayed sample: Day 5: 24.03%; Day 10: 23.46%; Day 15: 23.31%; and Day 20: 23.57%. In other words, the dilution of each raw milk sample was on the order of 1:4.

6. After data interpolation and back calculations accounting for the above sample dilution percentages the final prothrombin concentrations were determined for the following collection days:

    Day 5: 1.64 mg/ml (absolute intensity = 515562.80)
    Day 10: 1.67 mg/ml (absolute intensity = 513957.30)
    Day 15: 1.11 mg/ml (absolute intensity = 442443.95)
    Day 20: 0.79 mg/ml (absolute intensity = 402704.64)

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: October 6, 2010

Dr. William Velander

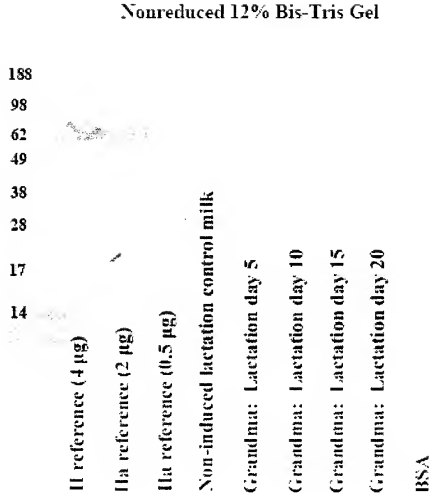
Nonreduced 12% Bis-Tris Gel
188
98
62
49
38
28
17
14
IL reference (4 µg)
IIa reference (2 µg)
IIa reference (0.5 µg)
Non-induced lactation control milk
Grandma: Lactation day 5
Grandma: Lactation day 10
Grandma: Lactation day 15
Grandma: Lactation day 20
BSA

Figure A

Create standard curve:

	ug	Mean Intensity	Std Dev	# Pixels	Absolute Intensity
Standard	0.5	84.64	4.89	3,698	312,998.72
	2	106.98	8.67	4,101	438,724.98
	4	115.45	7.36	4,382	505,901.90

$y = mx + b$

$m = 53,951.59$

$b = 302,313.43$

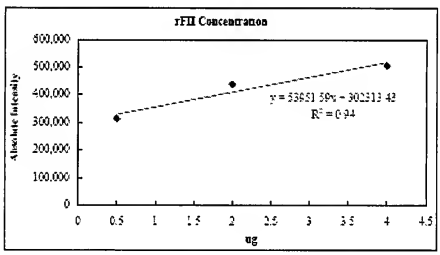

Figure B